date, however, intervention studies on the association between periodontal disease and CVD have been limited to observations on the effects of periodontal therapy on surrogate markers of risk for CVD or on pathways related to the pathobiology of the disease. The extent to which treatment of periodontal disease might result in lower incidence of CVD has not been addressed in any study so far.

Taking into account the strength and consistency of the association between periodontal disease and CVD, the overall benefits of good oral health, and the negligible risk associated with periodontal therapy, health education to promote better oral health should be considered as part of current healthy-lifestyle messages designed to reduce the burden of CVD in ESRD patients. However, further research is needed before specific recommendations are made regarding systematic monitoring and therapy of periodontal disease.

DISCLOSURE
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Neutrophils in acute kidney injury: not neutral any more

Subhashini Bolisetty[1] and Anupam Agarwal[1]

Awad and colleagues elucidate the spatiotemporal profile of neutrophil infiltration in the kidney following ischemia–reperfusion injury. Using elegant *in vivo* labeling techniques, they demonstrate increased neutrophil content in the kidney following ischemia–reperfusion, which is largely due to transmigration from the circulation into the interstitial compartment. The authors also provide mechanistic insights into this phenomenon and show that adenosine 2A receptor agonists reduce interstitial neutrophil infiltration and improve renal function.

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Neutrophils, the most abundant leukocyte population in circulation, are key effectors of the inflammatory cascade through their functional properties, which that include generation of reactive oxygen and nitrogen species, chemotaxis, and phagocytosis. The presence of increased neutrophils in the kidney has been described both in animal models and in biopsies from patients with acute kidney injury (AKI).[1] Such accumulation occurs particularly in the peritubular capillary network of the outer medulla as early as 30 minutes after ischemia–reperfusion.[2] Neutrophils adhere to endothelial cells with the help of specific adhesion molecules (intercellular adhesion molecule-1 and P-selectin) and, along with platelets and red blood cells, also cause capillary plugging, which leads to vascular congestion.[3,4] Degranulation of neutrophils; release of proteases, myeloperoxidase, and cytokines; and generation of reactive species can aggravate injury and damage endothelial and epithelial cells in the outer medulla.[5–7] However, the role of neutrophils in the pathogenesis of AKI has thus far been controversial.

Previous reports demonstrated that depletion or inhibition of neutrophil accumulation in the kidney during ischemia–reperfusion injury prevented AKI (reviewed by Jang and Rabb[5]). In addition, strategies to block neutrophil–endothelial interactions (for example, adhesion molecule antibodies) are protective in animal models of AKI.[8] In contrast, others showed no significant neutrophil accumulation during ischemia–reperfusion and neutrophil depletion did not protect from AKI.[9,10] Furthermore, nephrologists are often consulted for AKI in neutropenic patients in bone marrow transplant units, adding to the controversy about whether neutrophils are important in the pathogenesis of AKI.

These confounding results may be due to several reasons. First, the injury model used in each of these studies was different and not comparable. Variable times of ischemia may have influenced the severity of injury. Some studies used bilateral ischemia or right nephrectomy followed by unilateral ischemia. Recent work has shown that the systemic response to bilateral renal ischemia is greater than that to unilateral ischemia.[11] Second, the animal species used to study AKI were different. Some studies indicate that neutrophils accumulate in rodents (mice and rats) but not in rabbits following renal

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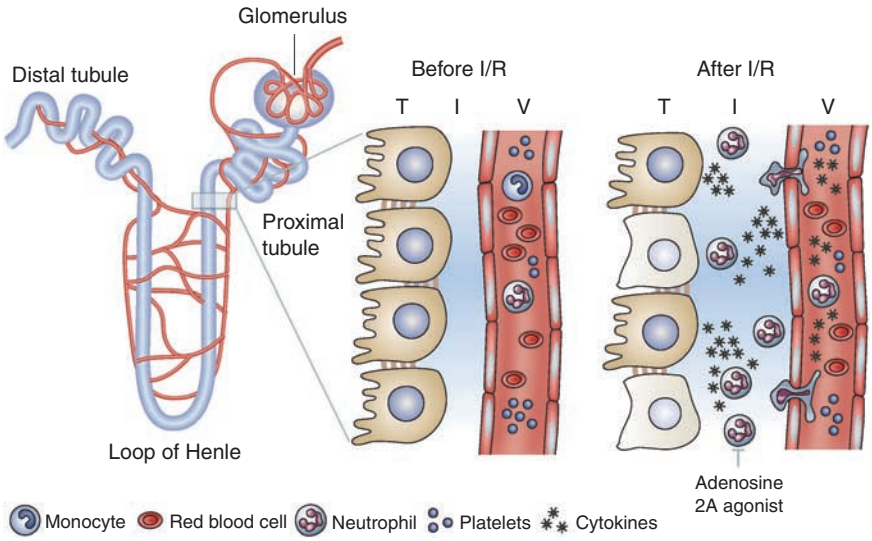

Figure 1 | Schematic showing the vascular (V), interstitial (I), and tubular (T) compartments in the outer medullary region before and after ischemia–reperfusion (I/R). After I/R, neutrophils transmigrate into the renal interstitium and, through the release of cytokines, chemokines, proteases, reactive species, and other products, can damage renal tubular epithelial cells and peritubular capillary endothelial cells. Adenosine 2A receptor agonists reduce neutrophil transmigration and improve renal function. (Adapted with permission from ref. 5)

ischemia–reperfusion injury.[10] Third, the method of neutrophil depletion was different in these models. Nitrogen mustard and antineutrophil serum were commonly used to deplete neutrophils. However, these strategies may not be completely effective in depleting the bone marrow and the marginating and transmigrated interstitial neutrophil pools. Thus, incomplete neutrophil depletion may have contributed to injury and confounded the results in identifying the role of neutrophils in AKI. Fourth, previous studies that showed a significant increase in neutrophil content in the kidneys did not identify the compartments where accumulation occurred. There have been reports that neutrophil compartmentalization had a substantial impact on the progression of injury in the lung. Finally, the method used for the detection of neutrophils may have also confounded the results. For example, the presumably neutrophil-specific detection methods using myeloperoxidase, naphthol chloroacetate esterase, or HIS-48 staining also crossreact with monocytes/macrophages.[12]

From all these studies, it is quite apparent that the role of neutrophils in AKI remained a conundrum until now. Awad and colleagues[13] (this issue) now provide a clear elucidation of the role of neutrophils in the pathogenesis of AKI induced by ischemia–reperfusion injury. One of the reasons for the limited analysis of neutrophil infiltration in previous studies was the lack of high-resolution imaging and specific markers that could be used to detect neutrophils. Using novel and quantitative flow cytometry-based and *in vivo* labeling techniques, the authors have identified the distribution and kinetics of accumulated neutrophils following AKI. They show that neutrophils accumulate in the kidneys following ischemia–reperfusion and transmigrate into the interstitium, which leads to increased vascular permeability, tubular epithelial and endothelial cell integrity, and aggravation of kidney injury (Figure 1). The interstitial neutrophils had a significantly different cytokine profile as compared with the marginating neutrophils, including a decrease in interferon-γ, interleukin-4, interleukin-6, interleukin-10, and tumor necrosis factor-α. The authors suggest that marginal neutrophils release cytokines during transmigration, which may account for the observed decrease in cytokines. The authors also tested the effects of bilateral versus unilateral ischemia–reperfusion and show that ischemic injury to one kidney does not increase interstitial neutrophil infiltration and vascular permeability in the contralateral nonischemic kidney.

Transmigration of neutrophils requires adhesion to endothelial cells via adhesion molecules such as P-selectin and intercellular adhesion molecule-1. Having shown that infiltrating neutrophils (not marginating neutrophils) are responsible for an increase in vascular permeability in the kidney, Awad *et al.*[13] chose to selectively inhibit transmigration using adenosine 2A (A_{2A}) receptor analogues. The A_{2A} receptor contains seven putative transmembrane-spanning domains, an extracellular amino terminus, a cytoplasmic carboxy terminus, and a third intracellular loop that is important in binding G proteins.[14] This receptor stimulates adenylyl cyclase and increases the production of cyclic adenosine monophosphate by coupling to stimulatory G proteins. In the kidney, A_{2A} receptor was found in the outer medulla (reviewed by Li and Okusa[6]). A_{2A} agonists bind to the A_{2A} receptors on endothelial cells and may inhibit neutrophil adhesion and transmigration by reducing the endothelial adhesion molecules. Although these agonists cause vasodilatation, the doses used by Awad *et al.*[13] do not have any effects on vascular tone. Previous work from the same laboratory showed that the degree of injury correlated with the neutrophil infiltration in mice ($r^2 = 0.73$; $P < 0.0001$) and rats ($r^2 = 0.94$; $P < 0.0001$) that underwent ischemia–reperfusion injury.[7]

Given the increasing interest in distant organ effects of remote ischemia,[15,16] Awad *et al.*[13] also addressed the effect of AKI on lung injury. Although an increase in lung neutrophil content was observed following AKI, no significant changes were noted in lung vascular permeability. The authors showed a significant increase in marginal neutrophils after AKI but observed no significant change in the lung interstitial neutrophil content. They propose that the lack of permeability changes in the lung after AKI was due to absence of interstitial neutrophils in the lung.

The results of Awad *et al.*[13] provide important mechanistic insights into neutrophil trafficking following renal ischemia–reperfusion injury and show that A_{2A} agonists reduce interstitial neutrophil infiltration in the kidney and

preserve renal function (Figure 1). Although A_{2A} agonists decreased the number of interstitial neutrophils, there was no change in the number of marginal neutrophils, indicating that this agonist is specific to transmigrating neutrophils. This specificity might be due in part to its ability to reduce the expression of adhesion molecules on the endothelial cells, limiting neutrophil adhesion, thereby inhibiting infiltration of neutrophils into the interstitium. Therefore, during ischemia–reperfusion, neutrophils transmigrate into the interstitium and cause an increase in vascular permeability and loss of kidney function. This process can be inhibited by blocking of neutrophil infiltration with A_{2A} receptor agonists, hence protecting against loss of kidney function in AKI.

In summary, the seminal findings of Awad *et al.*[13] highlight the importance of marginated versus interstitial neutrophils in the pathogenesis of AKI. The results have identified the pivotal role of the transmigrated neutrophil, which exhibits different phenotypic characteristics and is not just an innocent or 'neutral' bystander, since strategies to reduce this population of neutrophils lead to improved renal function in AKI.

DISCLOSURE

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